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TITLE OF THE INVENTION (280 characters max)				
METHOD FOR DISPLAYING MEMBRANE PROTEINS ON A LIPID BILAYER				
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PROVISIONAL PATENT APPLICATION

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METHOD FOR DISPLAYING MEMBRANE PROTEINS ON A LIPID BILAYER

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PATENT

METHOD FOR DISPLAYING MEMBRANE PROTEINS ON A LIPID BILAYER

BACKGROUND

The vast majority of therapeutics currently on the market target components of the cell membrane. A major challenge in discovering drugs against cell membrane targets is ensuring that isolated membrane samples and their components function as they do *in vivo*. When removed from their native cellular environment, cell membranes frequently lose their most physiological aspect: fluidity. Fluidity is one of the central membrane properties which facilitates the dramatic spatial rearrangement of receptors and signaling molecules during innumerable biochemical processes, ranging from intercellular communication to viral infection. For example, polyvalent ligands induce co-localization of their target receptors, thus encoding collective properties that are appreciatively different from individual binding events. In many cases, the ability of target receptors to move and adopt complimentary configurations is paramount to determining the overall affinity of the molecular recognition event. In G protein coupled receptor (GPCR) and integrin signaling, ligand binding triggers a conformational change in the receptor protein itself which, in turn, alters its association with other membrane signaling molecules. In each case, changes in the organization and mobility of membrane components occur in conjunction with signaling and recognition events.

In order to preserve the physiological integrity of cell membranes, most drug discovery programs have been forced to develop "live cell" assays for membrane targets. These approaches result in significant complexity and are often difficult to industrialize. Further, live cell systems make the mechanistic study of specific receptors highly problematic, as native cell membranes contain literally hundreds of unique receptors molecules.

U.S. Patent No. 6,228,326, incorporated herein by reference in its entirety, describes a system known as the MembraneChip™. This technology solves many of the above-described problems as it combines the physiological rigor of the *in vivo* environment with the high throughput requirements for industry drug screening. This technology consists of supported lipid bilayers elevated slightly above silicon substrates, and isolated from each other in discrete array corral spaces. The MembraneChip™ can

display cell membranes and their molecular components in a fluid and functional state characteristic of *in vivo* systems. This feature of the technology distinguishes it from other biological array technologies and facilitates an accurate representation of a myriad biological functions with unprecedented fidelity.

5 However, there remains a need for new methods of tethering membrane proteins to the above-described devices.

SUMMARY OF THE INVENTION

The present invention addresses this need. In particular, the invention provides methods of generating and displaying extracellular and intracellular domains of transmembrane proteins on lipid bilayer arrays, such as those arrays described in U.S. Patent Nos. 6,228,326 and 6,503,452; U.S. Patent Publication Nos. 20020009807 and 20020160505; and PCT Publication No. WO 01/26800, all of which are incorporated herein by reference in their entireties. Particularly preferred is the use of the present invention with the fluid bilayer membranes described in U.S. Patent No. 6,228,326. The ability of the MembraneChip™ described therein to display characteristics of living cell membranes greatly facilitates its use for the study of membrane proteins which regulate nearly every aspect of cellular physiology.

The methods described herein provide membrane arrays for use in applications including simple quality assurance/quality control optimization studies, medical diagnostics, and drug discovery involving cell-cell interactions. Intracellular domains displayed using these methods enable investigation of whole intact signal transduction pathways. The method of display described herein, which can handle the majority of membrane proteins in the genome containing a discrete functional intracellular or extracellular domain, enables a myriad of applications in both industry and the medical clinic.

For example, receptor tyrosine kinases (RTKs), which are critical mediators of cellular growth and differentiation and have been associated with various cancers, are greatly amenable to targeting using the present technology. The ligand-induced dimerization and autophosphorylation characteristic of RTKs can faithfully be reconstituted for drug discovery given the fluidity of the MembraneChip™ environment. In addition to studying membrane proteins regulating cell autonomous events, the technology is useful for modeling intercellular interactions, including cell-cell adhesion and trans-cellular signal transduction. Cell-cell signaling is critical for regulating cellular proliferation and differentiation, the development of organs and tissues during embryogenesis, the establishment of neuronal synapses, vasculogenesis, and the activation of an immunological response. Fundamentally, these intercellular interactions occur through the binding of cognate membrane-bound ligands and receptors, initiating

signal transduction cascades in the apposing cells. Inappropriate cell-cell communication has been implicated in a number of disease states, including cancer and autoimmunity, making the associated signaling molecules important targets for drug discovery efforts. Arraying a membrane-bound ligand in a chip corral and overlaying with an intact cell expressing its membrane-bound receptor combines the advantages of having a well-defined, purified component and being able to study a complete signal transduction pathway simultaneously.

In the context of the immune system, for example, displaying antigen peptide complexed to MHC and accessory adhesion and co-stimulatory molecules in a fluid bilayer can successfully recapitulate the activity of antigen-presenting cells. Overlaying intact T cells specific for the antigen peptide displayed on the chip enables their rapid activation, facilitating the targeting of nearly every checkpoint in the T cell activation pathway (see, e.g., Grakoui et al., *Science* (1999) 285:221-227, for a discussion of this interaction).

These and other embodiments and uses of the subject invention will readily occur to those of skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the expression vector pYBS101 used in the examples herein.

Figures 2A-2C show FACS analysis of the experiment described in the examples using ICAM-1 (Figure 2A) and B7-1 (Figure 2B), as well as an SDS-PAGE analysis (Figure 2C) of ICAM-1 and B7-1.

Figures 3A-3B show the results of experiments to determine the functionality of MembraneChips™-displayed proteins.

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of molecular biology, chemistry, biochemistry, recombinant DNA techniques and immunology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., Blackwell Scientific Publications); A.L. Lehninger,

Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.).

All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entireties.

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

The present invention is based on the discovery of a method for displaying the extracellular and intracellular domains of transmembrane proteins by anchoring the domains to a fluid bilayer membrane, such as, but not limited to, those present in the MembraneChip™ described in U.S. Patent No. 6,228,326, incorporated herein by reference in its entirety. Particularly, membrane proteins are tethered to fluid bilayer membranes using, for example, GPI anchorage, other lipid attachements (e.g. myristoylation or palmitoylation), avidin/streptavidin/neutravidin protein fusions binding to biotinylated membrane lipids, hexa-histidine-tagged proteins tethered via Ni^{2+} membrane coordination, glutathione-S-transferase fusion proteins binding to membrane lipids conjugated with glutathione, and maltose-binding protein fusion proteins binding to membrane lipids conjugated with maltose.

Membrane proteins constitute a significant proportion of total proteins in the human genome, and have been shown to regulate cellular processes as diverse as growth and differentiation, cell-cell adhesion, neuronal synaptogenesis, and immune cell activation. It is estimated that of the almost 20,000 well-characterized human proteins (NCBI Human Reference Protein Sequence, 9/10/03), approximately 24.3% are predicted to have transmembrane segments by the TMHMM algorithm (Technical University of Denmark). While 2,658 of these are predicted to have a single transmembrane domain, a

large number of the 531 human proteins predicted to have two transmembrane domains likely span the membrane only once, as the hydrophobic nature of the signal peptides make them often register as transmembrane segments on routine database searches. Approximately 0.5% of these signal sequencing-bearing proteins are predicted to already contain a GPI anchor, a naturally occurring membrane tether. In essence, the present invention enables the use and study of over 66% of the membrane proteins, as an additional protein category including proteins with multiple transmembrane domains, but with discrete extracellular ligand binding domains and intracellular signaling domains, have not been accounted for in the above estimate. Consequently, the universal method of protein expression and display described herein, facilitates discovery and research pertaining to both the extracellular and intracellular aspects of the vast majority of membrane proteins.

The physiological qualities of the MembraneChip™ make it extremely useful for studying processes requiring intercellular contact. For example, GPI-linked extracellular domains of single-pass membrane proteins displayed in the supported lipid bilayer can serve as adhesion molecules and signaling ligands for cognate receptors in overlaid intact cells, enabling growth of various tissues. Numerous studies have suggested that stem cells can different into various cell types *in vitro* through co-culturing with specific tissues (Prockop et al., *Proc. Natl. Acad. Sci. USA* (2003) 100:11917-11923). Accordingly, the membrane arrays of the present invention can facilitate the generation of organ tissue on an industrial scale. Thus, by providing minimal signals required for differentiation of stem cells into a particular tissue present in the extracellular domains of membrane proteins, stem cells can be cultured in the context of a MembraneChip™ displaying the extracellular domains of these proteins. Indeed, growing human tissue which is suitable for transplantation or implantation into a diseased organ would be a major advance in public health, particularly given that approximately 73,000 people nationally are currently awaiting an organ transplant and 10,000 die annually due to the shortage of cadaveric organs.

Intercellular signaling is of fundamental importance to cancer, as tumors often require intimate contact between cells for their growth and maintenance. The single-pass erythropoietin producing hepatoma (Eph) receptors (EphRs) are the largest family of

RTKs, and have been shown to be important for carcinogenesis (Dodelet et al., *Oncogene* (2000) 19:5614-5619). Moreover, the ligands of EphRs, known as Ephrins, are either single-pass or GPI-anchored membrane proteins (Cutforth et al., *Trends Neurosci.* (2002) 25:332-334), reflecting the critical role of cell-cell contact for transmembrane signal transduction by this receptor family. EphRs/Ephrin signaling has a fundamental role in the process of angiogenesis, the elaborate program by which tumors become vascularized in order maintain an oxygenated state. Angiogenesis thus requires intimate cell-cell interactions and signaling, and has also been a key mechanistic target for therapeutic intervention for cancer (Huang et al., *Proc. Natl. Acad. Sci. USA* 100:7785-7790). Using the methods of the present invention, the extracellular domains of the EphRs or Ephrin ligands can be displayed, and intact signal transduction pathways through their cognate receptors on overlaid intact cells can be measured. In the case of cancer, the appropriate EphR/Ephrin pairing mediating angiogenesis can be studied for purposes of identifying drugs which perturb the signaling pathway. Consequently, this method of displaying single-pass membrane proteins enables the identification and validation of novel cancer chemotherapeutics.

In addition to tissue regeneration and cancer, the process of immunological activation requires extensive intercellular interaction and signal transduction. The sequential stages which ultimately lead to cytokine release and proliferation of activated T cells necessitate intimate association with APCs. In the case of the T cell/APC interaction, the cell-cell contact is extremely tight as to be referred to in "synaptic" terms, and greatly facilitates the precise patterning of T cell receptor, MHC, adhesion, and co-stimulatory molecules which mediate the immune response (Grakoui et al., *Science* (1999) 285:221-227). Moreover, supported lipid bilayers displaying ICAM-1 in the context of MHC and specific antigen peptide have been shown to be sufficient for immune synapse formation and bone fide activation of overlaid intact T cells (Grakoui et al., *Science* (1999) 285:221-227). As MHC molecules, ICAM-1, and the majority of other adhesion and co-stimulatory molecules are either single-pass or GPI-anchored membrane proteins, the present method of displaying such proteins affords a powerful opportunity for studying immune system activation. Further, as execessive T cell activation underlies a number of severely debilitating autoimmune diseases, including

multiple sclerosis and Crohn's disease, this technology can lead to better treatments for these immunological disorders.

The present invention also facilitates the study of intracellular signaling through display of the cytoplasmic domains of membrane proteins. RTKs are one particular family of receptors whose intracellular signal transduction pathways are potential targets for anti-cancer therapy. The epidermal growth factor receptor, perhaps the most well-known RTK, is upregulated in a number of cancers (Gill et al, *Mol. Cell Endocrinol.* (1987) 51:169-186), and is the target for the antibody therapeutics Erbitux and Herceptin. As the cytoplasmic kinase domains of RTKs have been shown to have constitutive activity when separated from their extracellular and membrane-spanning domains, it may be possible to reconstitute the signal transduction pathway emanating from an intracellular domain tethered to a fluid bilayer, such as present on the MembraneChip™. In this embodiment, the intracellular domain of an RTK is anchored to the MembraneChip™ through an N-terminal membrane anchor, followed by incubation with cell extracts or purified components of the signaling pathway. In this way, *in vitro* reconstitution of an RTK pathway facilitates the identification of small molecule drugs which can be screened for incredible specificity. Various RTKs can be arrayed on the MembraneChip™ in the presence of common signaling machinery, allowing for identification of inhibitors which are specific for a given RTK. Target RTKs are arrayed in parallel with non-target RTKs, enabling the identification of highly-specific small molecule drugs affecting tyrosine kinase pathways and possibly active against various cancers.

The present method for membrane protein display is also particularly enabling for medical diagnostics. Infectious disease is becoming an increasingly important branch of medicine, and the ability to monitor the effectiveness of vaccinations against various pathogens has become extremely valuable. Commonly, vaccination results in the production of T cells specific for the particular antigen being administered. By displaying an MHC molecule complexed with a pathogenic antigen, it is possible to isolate T cells specific for that antigen from a patient's blood serum. In this embodiment, a patient vaccinated against a whole panel of pathogens supplies a simple blood sample which is mixed with the same panel displayed in MHC on the fluid bilayer array,

facilitating a single high-information content screen for responding T cells indicative of a successful vaccination. Displaying MHCs with antigen is also beneficial for diagnosing various autoimmune diseases. For example, self-antigens which have been implicated in multiple sclerosis can be displayed on a chip in order to test for the presence of the specific population of T cells thought to be responsible for the disease pathology.

The present method also provides a powerful device for quality assurance and control applications. Drugs, small molecules, antibodies, biologicals and the like, which have been identified to have activity against any of the membrane proteins displayed using this method, are easily characterized and optimized using this technology. The array format of the technology easily facilitates studies of specificity and sensitivity, and is compatible with a number of different detection systems, including our label-free approach and surface plasmon resonance. In this manner, affinity measurements for active drugs are obtained very rapidly, facilitating further optimization and validation. Consequently, the present method of membrane protein display is extremely advantageous for a number of different fields.

Single-Pass Membrane Proteins in the Immunological Synapse		
	APC	T Cell
Signaling Molecules	MHC I MHC II	TCR CD4 CD8 CD3
Co-stimulatory Molecules	B7-1 (CD80) B7-2 (CD86) ICOSL CD40 PD-1	CD40L CD28 ICOS PD-L1 PD-L2 4-1BB (CD137)
Adhesion Molecules	ICAM-1 LFA3 (CD58)	LFA1 CD2

EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Materials and Methods

Cells.

CHO, HEK-293, and Jurkat T cells were obtained from the University of California, San Francisco Cell Culture Facility and cultured in Ham's F12, Dulbecco's Modified Eagle Medium, and RPMI 1640 media, respectively, in 37 °C humidified incubators with 5% CO_2, according to manufacturer's recommendations.

Generation of GPI Expression Vector.

pYBS101 was generated through modification of pcDNA3.1(+) (Invitrogen). The Igκ leader sequence followed by sequence encoding six tandem histidine residues was cloned into *NheI-Hind*III-digested pcDNA3.1(+) as an oligonucleotide, regenerating only the *Hind*III site. Sequence encoding the 32 terminal amino acids of the GPI-anchoring sequence from placental alkaline phosphatase was PCR amplified from pVac2-mcs (Invivogen) as an *Xba*I-*Nhe*I fragment and ligated into the *Xba*I site of pcDNA3.1(+) with the previously mentioned modification, regenerating the *Xba*I site only at the 5' end of the sequence, generating pYBS101.

Expression of Membrane Proteins in GPI-anchored form.

cDNAs encoding the extracellular domains (excluding signal sequences) of human B7-1, ICAM-1, and CD58 were amplified from ESTs (Invitrogen) and cloned into pYBS101 as *Hind*III-*Xba*I fragments with enterkinase-encoding cleavage sites (DDDDK)

at their N-termini. CHO cells were transfected with expression plasmids using calcium phosphate transfection (Promega) or SuperFect (Qiagen) following manufacturers recommendations. 48 hours after transfection, cells were split at 1:5, 1:10 and 1:20 into growth media containing 500 μg/ml of G418 (Invitrogen) for generation of stable lines expressing human B7-1 and ICAM-1. Polyclonal stable cell lines were maintained and passaged in G418-containing media for 4 weeks. B7-1 and ICAM-1 were also expressed transiently in HEK-293 cells. Expression of these proteins was confirmed by FACS analysis using PE-conjugated antibodies (Beckton Dickinson).

Purification of Membrane Proteins.

Confluent CHO cells from 6-8 10-cm dishes were washed 2X with cold PBS, and were scraped into 600 ul (per dish) of lysis buffer containing 50 mM Tris-Cl pH 8.0, 150 mM NaCl, and 1 % Triton X-100, and a protease inhibitor cocktail (Sigma). Harvested cells were solubilized on ice for 30 minutes, followed by microcentrifugation for 15 min at highest speed. Ni-NTA agarose (Qiagen) was washed 1X with lysis buffer and added to clarified extracts, 50 μL of beads per 600 uL of cell extract. Cell extracts were incubated with beads for 2 hours with rotation at 4° C. Beads were washed 4X with buffer containing 50 mM Tris pH 8.0, 150 mM NaCl, 10 mM imidazole (Sigma), and 1% Octyl-Glycopyranoside (Calbiochem) plus a protease inhibitor cocktail. Bound proteins were eluted with 600 ul of buffer containing 50 mM Tris pH 8.0, 150 mM NaCl, 200 mM imidazole, and 1% Octyl-Glycopyranoside plus a protease inhibitor cocktail for 1 hour at 4 °C with rotation. Eluted proteins were approximately 50% pure as examined by SDS-PAGE and was approximately 6.8 μg B7-1/mg total protein and 3.3 μg ICAM-1/mg total protein. according to Bradford analysis (BioRad). Approximately, 10-50% of purified protein remains bound to the beads following elution, suggesting that actual purification yields could be improved somewhat through optimization of this step.

Reconstitution of Membrane Proteins.

Aliquots of protein extracts were added to an EggPC lipid mix (1% NBD-PG, 99% EggPC) and dialyzed overnight at RT against PBS, with 3X buffer changes. While egg PC was used to generate membranes in this case, these proteins could easily be

reconstituted in vesicles with various lipid compositions. To form the supported lipid membranes, 20 μL of undiluted (or diluted 1:2) protein-lipid mix was deposited onto MembraneChips™ or cover slips for 3-5 min at RT, followed by 5 washes with buffer. Incorporation of protein into the supported membrane was confirmed by immunofluorescence using antibodies to the respective. Membranes were stained with 30 μl of PE-conjugated antibodies (BD) in 5% FCS for 30 min then washed extensively with PBS and assessed by fluorescent microscopy. Protein densities are approximately 10,000-20,000 molecules/μm^2, as estimated from a proteolipid concentration of 0.6 μg/μL (50% pure) in a 20-μL drop on a chip surface of 7 mm diameter. It was assumed that only 1% of the drop contents are retained on the chip surface for membrane display.

Jurkat Adhesion to MembraneChip™.

Cell adhesion assays were performed as previously described with several modifications (Chan et al., *J. Cell. Biol.* (1991) 115:245-255). Briefly, membranes were blocked with 10% fetal bovine serum (VWR) in PBS for 1 hour at RT, followed by incubation for 10 minutes with RPMI 1640 media (Invitrogen) containing 10% FBS at 37 °C. 1×10^5 Jurkat T cells were added to the well for 15 minutes. The approximate number of cells per membrane corral was determined by counting under brightfield microscopy (usually 150-200 cells/coral). MembraneChips™ were subsequently inverted into a dish containing 10% FBS in PBS for 15-20 minutes to wash unbound cells. The final cell number was determined by counting under microscope directly or after taking images. In experiments where monoclonal antibodies against human B7-1 or ICAM-1 (Santa Cruz) were used to block Jurkat T cell adhesion, MembraneChips™ were pre-incubated with antibody followed by 3X washes with PBS prior to incubation with cells.

Results

Method for Displaying Membrane Proteins in MembraneChip™.

A protocol for expressing and displaying membrane proteins in the MembraneChip™ supported bilayer technology was developed. For proof-of-concept

experiments, plasmid pYBS101 was constructed. This is a versatile expression vector made by modifying pDNA3.1(+) (Fig. 1) to contain a 5' signal sequence and hexa-histidine epitope tag and a 3' GPI-anchor sequence.

While the experiments reported herein made use of the signal sequence from the Igκ chain given that it is very well-characterized, the signal sequence from nearly every secreted protein and the majority of membrane proteins may be used instead. Examples of secreted and membrane proteins containing signal sequences include epidermal growth factor (secreted), insulin (secreted), nerve growth factor (secreted), platelet-derived growth factor (secreted), glucagon (secreted), ICAM-1 (membrane protein), B7-1 (membrane protein), TrkA (membrane protein), platelet-derived growth factor receptor (membrane protein), and CD58 (membrane protein). Additionally, epitope tags other than hexa-histidine may be used, such as myc, Flag, HA, gluthathione-S-transferase, and maltose-binding protein.

Methods for tethering protein domains to membrane arrays, other than GPI anchorage include other lipid attachements (e.g. myristoylation or palmitoylation), avidin/streptavidin/neutravidin protein fusions binding to biotinylated membrane lipids, hexa-histidine-tagged proteins tethered via Ni^{2+} membrane coordination, glutathione-S-transferase fusion proteins binding to membrane lipids conjugated with glutathione, and maltose-binding protein fusion proteins binding to membrane lipids conjugated with maltose.

cDNAs encoding the extracellular domains of transmembrane proteins of interest are cloned into the flexible multiple cloning site of pYBS101. Optionally, cDNAs may be subcloned with an enterokinase-cleavage site at the 5' end, providing the ability to remove the His tag if necessary. Other proteases and protease-recognition sequences can also be used instead of enterokinase, including but not limited to, thrombin and Factor Xa. While pYBS101 currently has a neomycin resistance gene for selection of stably-transfected cells, the vector is easily modified to enable selection in a number of other drugs including, but not limited to, hygromycin and zeocin. This added flexibility facilitates selection of transfected cells co-expressing different proteins, such as a multimeric complex.

Transfection of the resulting construct into mammalian cells such as CHO or HEK-293 generates an extracellular domain targeted to the plasma membrane and tethered by a C-terminal GPI anchor. Expression in mammalian cells can also be accomplished using retroviral vectors, depending upon how successful the standard transfection reagents are for any given protein. The fusion of an N-terminal hexa-histidine sequence facilitates rapid and high-efficiency purification on Ni^{2+} resins. While the examples herein relate to the generation and display of the extracellular domains of membrane proteins, the technology can easily accommodate incorporation of intracellular domains of membrane proteins. In this embodiment, an expression plasmid analogous to pYBS101 is fabricated with a 5' signal sequence for myristoylation and a 3' hexa-histidine epitope tag. Using this vector, cDNAs encoding the intracellular domains of membrane proteins are subcloned into the multiple cloning site flanked by these two modification sequences. Expression in mammalian cells generates an intracellular domain tethered to the membrane by myristoylation which is easily purified by the Ni^{2+} resins.

Validation of Membrane Protein Display.

The pYBS101 expression system was validated using two proteins of the immunological synapse which mediate the interaction of T cells with antigen-presenting cells: B7-1 (~240 aa., ~27 kDa) and ICAM-1 (~487 aa., ~54 kDa). cDNAs encoding the human isoforms of these proteins were cloned into pYBS101 and expressed either stably (in CHO cells) or transiently (in HEK-293 cells). FACS analysis (Fig. 2A and 2B) demonstrated a high level of expression of these proteins, as well as their correct targeting and tethering to the plasma membrane (untransfected on left, transfected on right). Purification of these proteins was accomplished in one step using Ni^{2+} resins, and SDS-PAGE indicates proteins were of the predicted size (Fig. 2C). The purified proteins were subsequently reconstituted into egg phosphatidylcholine vesicles and arrayed into membrane corrals of the MembraneChip™.

Immunofluorescence microscopy revealed the presence of these proteins in the supported bilayers. In cases where it would be desirable to release soluble protein from

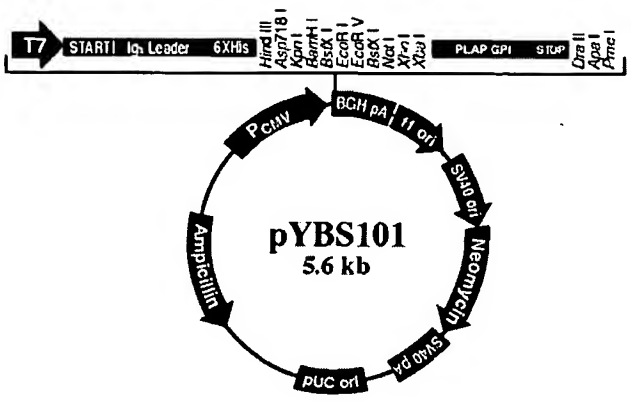
the chip array, enzymatic cleavage of the GPI anchor with phosphatidylinositol phospholipase C can be accomplished.

Jurkat T Cell Adhesion Assay.

In order to demonstrate that the purified, chip-displayed proteins were functional, the ability of the system to mediate Jurkat T cell adhesion was tested (Fig. 3A). Approximately 1×10^5 Jurkat T cells were incubated with MembraneChips™ with or without displayed human B7-1 or ICAM-1 at densities of approximately 10,000-20,000 molecules/μm^2. Robust Jurkat T cell adhesion was observed only to membranes displaying these proteins, as the cells do not adhere nearly as well to membranes devoid of protein. As a further test of the specificity of the cell adhesion, we attempted to block the CD28/B7-1 and LFA-1/ICAM-1 interactions with monoclonal antibodies against the extracellular domains of human B7-1 and ICAM-1, respectively. The results depicted in Fig. 3B demonstrate that pre-incubating membranes with antibodies against B7-1 or ICAM-1 abolished Jurkat T cell adhesion to MembraneChips™ displaying the respective proteins, reducing the cell adhesion to background levels observed in membranes devoid of protein. This latter result demonstrates the great applicability of the assay to drug discovery, given the potential for therapeutic intervention with specific monoclonal antibodies at this stage of T cell activation for disorders ranging from autoimmunity to cancer.

Thus, novel methods of displaying extracellular and intracellular domains of membrane proteins on fluid bilayer membranes are described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined herein.

Figure 1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BamH I
BstX I
EcoR I
EcoR V
BstX I
Not I
Xho I
Xba I
PLAP GPI
STOP
Cla II
Apa I
Ama I
P CMV
BGH pA
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SV40 ori
Neomycin
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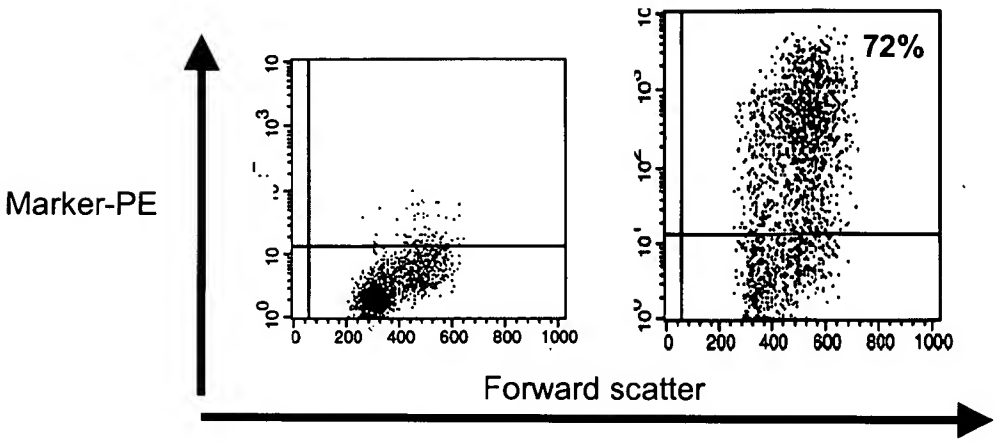
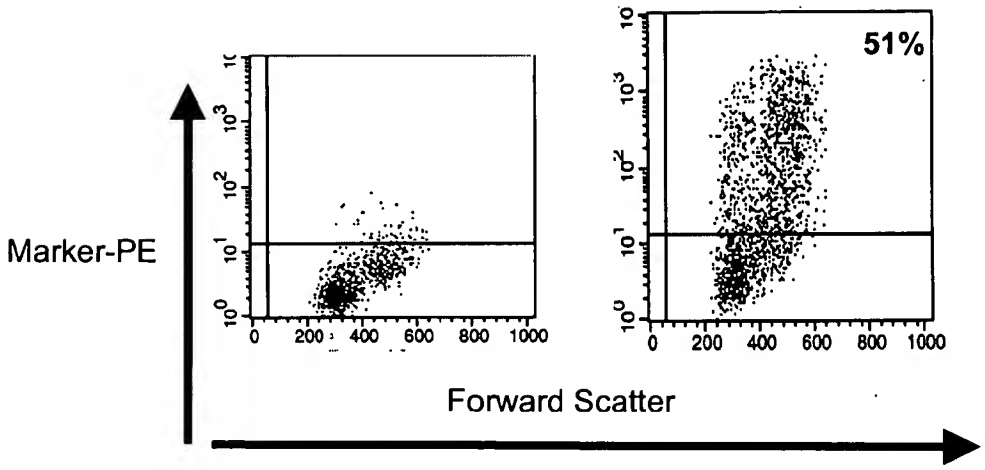
PUC ori
Ampicillin
pYBS101
5.6 kb

Figure 2

A ICAM-1



B B7-1



C

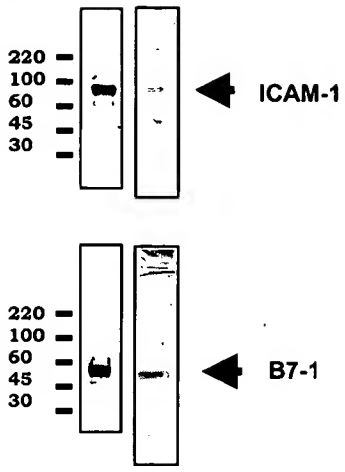
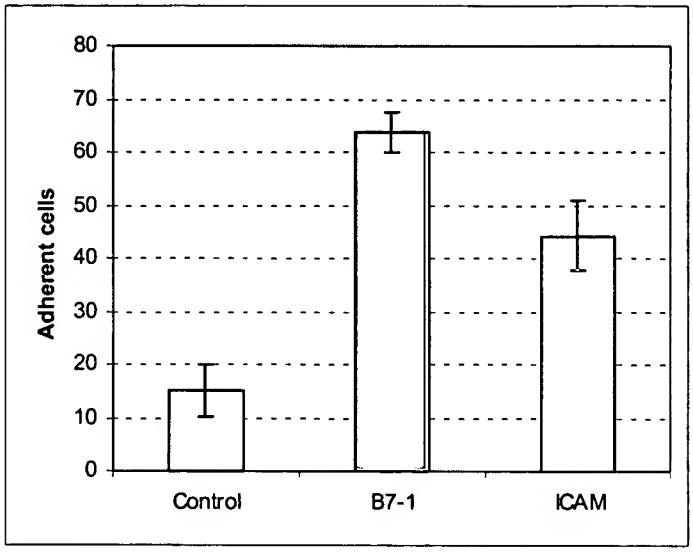
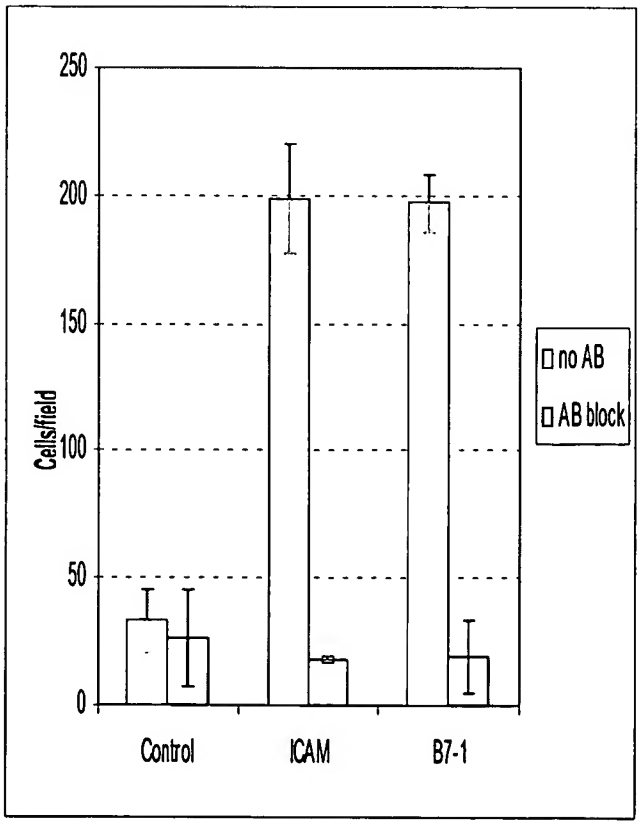


Figure 3

A


80
70
60
50
40
30
20
10
0
Adherent cells
Control
B7-1
ICAM

B


250
200
150
100
50
0
Cells/field
Control
ICAM
B7-1
no AB
AB block